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Daytime unresponsiveness of the human and Syrian hamster pineal to adrenergic stimulation

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SUMMARY

The human melatonin rhythm is remarkably stable in the face of many other endocrine and metabolic disturbances. Daytime induction of sympathetic activity and injection of β-agonists do not stimulate levels of circulating or excreted melatonin in humans. This seems paradoxical in that the human nocturnal surge in blood and urinary melatonin does depend on sympathetic innervation to the pineal. The gland is sensitive to β-adrenergic stimulation at night; β-blocking drugs can eliminate the nocturnal melatonin surge in humans. The Syrian hamster has been developed as a model for the human findings. Its nocturnal melatonin surge is blocked by β-blockade or by pineal denervation. Injection of norepinephrine (NE) or isoproterenol (ISO) during the second half of the dark phase (after interruption of the endogenous melatonin surge by short light exposure) raises pineal melatonin content; injection outside this sensitive period does not. This dramatic change in response of the pineal gland to its neurotransmitter between day and night has been documented by in vitro methods and is temporally associated with an unexpected fall in membrane β-receptor ligand binding only during the sensitive period. Though the mechanisms for the rhythm of responsiveness within the pineal are yet to be fully elucidated, daytime unresponsiveness may contribute to the stability of the melatonin rhythm.

KEY WORDS
Pineal, melatonin, human. hamster, adrenergic stimulation

MELATONIN IS NOT AN INDEX OF GENERAL SYMPATHETIC ACTIVITY

The stability of the human melatonin rhythm - in the face of traumatic bodily injury, pituitary disease, or central nervous system lesions outside a specific neuronal pathway controlling the pineal - has been appreciated (Vaughan, 1984; 1986). We proposed that this stability in the presence of many acute and chronic adverse stimuli imparts to the melatonin rhythm a characteristic that would be important for a signal that represents relatively specifically the underlying biological clock as it has been set by the light-dark cycle in the days prior to measurement. Other hormonal signals seemed more pertubable by extraneous influences. Recent reports suggest little (Webley and Leidenberger, 1986; Kivela et al., 1988) or no (Berga and Yen, 1988; Brzezinski et al., 1988) detectable effect even of the menstrual cycle on the melatonin rhythm, though seasonal effects

have been described (Beck-Friis et al., 1984; Kauppila et al., 1987; Kivela et al., 1988). Mild blunting of the serum melatonin nocturnal surge in severely burned patients (Vaughan et al., 1985) and augmentation of the surge in spontaneously amenorrheic women (Berga et al., 1988; Brzezinski et al., 1988) may represent altered hypothalamic function, known to occur in these conditions. The pathway transmitting the neural signal to the pineal for the nocturnal surge passes through the hyothalamus (Vaughan, 1984; 1986). Even in these conditions, there was no major abnormality in the timing of the peak. Previous suggestions of a reduced melatonin surge in psychiatric depression have been questioned by recent results (Thompson et al., 1988).

The most consistent artificially induced changes in the human melatonin rhythm have been those of re-entrainment of the surge to the dark period over several days after changing the light-dark phasing (see Vaughan, 1984), and acute suppression of the nocturnal surge with strong light (Lewy et al., 1980; Strassman et al., 1987). Of course, lesions that destroy elements of the neural pathway to the pineal reduce or eliminate the nocturnal surge of melatonin in blood and urine (Vaughan, 1984; Tetsuo et al., 1981; Li et al., 1988).

Experimental animals have also been variably resistent to induction of changes in the melatonin rhythm if the light-dark cycle is maintained unchanged in the absence of neural lesions in the animals. The "flow" phase of severe injury (e.g., days to weeks after a major skin burn) is characterized by many striking hormonal and metabolic aberrations, including elevated sympathetic tone, though the rhythm in pineal melatonin content in burned rats and hamsters is essentially normal (Vaughan et al., 1985). Acute adverse stimuli, such as insulin-induced hyoglycemia (Lynch et al., 1973; Champney et al., 1985) and forced swimming (Parfitt and Klein, 1976) applied during the light phase raise pineal melatonin content in rats, though the change is small in rats with functional pineal nerve endings. Ether anesthesia (G.M. Vaughan and J.P. Allen, unpublished results) and forced swimming (Troiani et al., 1988) produced a paradoxical reduction of the high nocturnal pineal melatonin values when these stressors were applied at night in the rat. Such paradoxical acute results at night are poorly understood. In the Syrian hamster, insulin-hypoglycemia during the daytime produced no rise in pineal melatonin content (Champney et al., 1985). The latter finding is consistent with absence of stimulation of circulating or excreted melatonin in humans during the day by a variety of influences (including naps, psychological stress, exercise, insulin hypoglycemia, and painful diagnostic procedures) some of which provoke intense responses in the sympathetic and other hormonal systems (see Vaughan, 1984; 1986 for review).

Best exemplified so far in humans and hamsters, the stolid nature of the melatonin rhythm appears paradoxical with respect to the pineal's innervation by the sympathetic nervous system, a system generally noted for mediating massive end-organ responses to many stimuli. Instead, for the pineal, its sympathetic noradrenergic innervation apparently represents a selective channel of signals not reflecting general sympathetic activity in the ordinary sense, but rather reflecting the timing of the previous pattern of dark periods in the light/dark cycle (Vaughan 1984, 1986).

DAY-NIGHT DIFFERENCE IN PINEAL RESPONSIVENESS

Aside from experiments with induction of systemic endogenous sympathetic activity, others employing more specific stimulation of the pineal or administration of adrenergic agents have also been informative. They add another dimension to the picture: alteration of the response to catecholamine in the pineal itself during the night. A greater response during the night (versus during the day) is seen in the rat, and this day-night difference within the pineal is more profound in hamsters and humans, further suggesting that the Syrian hamster may be a better

model for the human in regard to neural control of the melatonin rhythm.

Electrical stimulation of the cervical sympathetic trunk in the rat produces a rise in pineal N-acetyltransferase activity (NAT, a reflection of melatonin synthesis) during the daytime, but the response is much quicker at night (Bowers and Zigmond, 1982). Since administration of β-antagonists blocks the nocturnal melatonin surge in mammals, including humans (Vaughan, 1986), pharmacologic attempts at stimulation of daytime melatonin synthesis have involved use of β-agonists, principally ISO or the neurotransmitter itself, NE. One complication with the use of NE (but not ISO) injections is that pretreatment with a nerve ending uptake-blocker is required for a melatonin response of any appreciable magnitude, since the injected NE may accumulate in sympathetic endings rather than reach post-junctional sites of action on pinealocytes (Parfitt and Klein, 1976; Vaughan et al., 1986). One advantage of using NE is that it includes some alpha activity which may augment the predominant β-stimulation of melatonin synthesis at night (Santana et al., 1988).

Injection of ISO into rats during the day produces a rise in pineal and circulating melatonin (Wu et al., 1987). Injection of several β-adrenergic agonists, including ISO, or administration of L-dopa, during the daytime in humans fails to raise circulating melatonin (see Vaughan, 1984; 1986 for review). Similarly, injection of ISO during the day in intact or pineal-denervated hamsters produces no rise in pineal melatonin (Lipton et al., 1982). Though one study showed prevention (by ISO injection) of the nocturnal fall in hamster pineal melatonin otherwise seen after acute light exposure at night (Steinlechner et al., 1985), the marked unresponsiveness of Syrian hamster melatonin to catechol injection during the day (Binkley, 1976; Steinlechner et al., 1984) raised the specter of doubt about sympathetic control of the melatonin rhythm in humans and Syrian hamsters. However, we reasoned that the previously observed prevention of the nocturnal melatonin surge by β-blockade in both species and the prevention of the acute light-induced fall in pineal melatonin by ISO in hamsters indicates that the pineal becomes sensitive to the neurotransmitter at night: in the proper circumstance, experimental stimulation by catechol should raise pineal melatonin and thus support the role of the sympathetics in controlling the melatonin rhythm.

After male Syrian hamsters were exposed to a short period of light (acutely to lower pineal melatonin prior to injection) in the second half of the dark phase, injecting 1 mg/kg of NE (10 min after injection of desipramine to block the protective neuronal uptake system) produced a dramatic rise in pineal melatonin not seen after NE injection alone (Vaughan et al., 1986). Additionally, when pineals were taken at night for direct incubation with these agents, 1 μM NE in the medium (potentiated by desipramine) stimulated melatonin synthesis. Equivalent experiments done near the middle of the light phase showed no stimulation of melatonin in vivo or in vitro. Figure 1 shows that ISO injection alone after short light exposure in the second half of darkness raised hamster pineal melatonin; whereas, the same dose at the end of the light phase did not. Other results (Vaughan et al., 1986; Reiter et al., 1987a; 1987b) showed that NE or ISO injections in vivo or incubations in vitro were effective only during a sensitive period during and slightly beyond the second half of the dark phase. The maximal response in vivo was 2 h after injection. Light exposure during the first half of the night prevented development of normal pineal responsiveness in hamsters. Figure 2 shows the dose-response relationship with ISO in vitro for rats and hamsters at the end of the light phase and in the second half of the dark phase after 30 min exposure of the animals to light. Melatonin in the medium (93-97% of all the melatonin present) after 4-h incubations of single pineals essentially represents new melatonin synthesis. Rat pineal responsiveness was present at the end of the day but greater at night. In the hamster, it was not demonstrable at the end of the day but present at night.

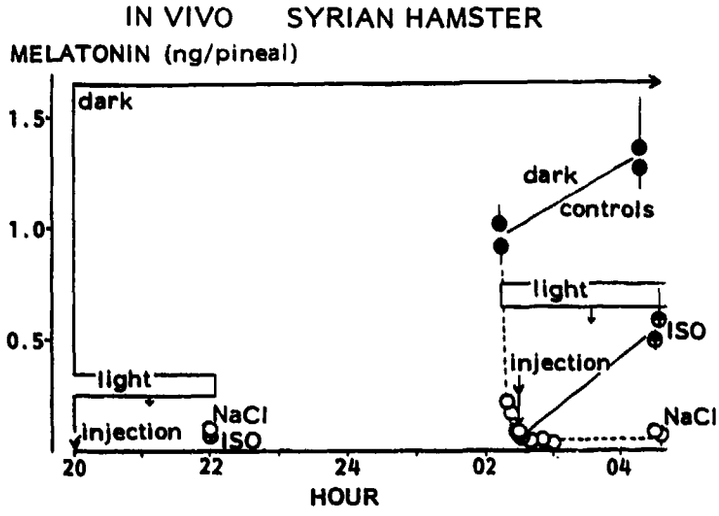

Fig. 1. Mean ± SE response to isoproterenol (ISO, 1 mg/kg) injected sc once at the end of the usual light phase or once in the second half of the usual 10-h dark phase (redrawn from Vaughan and Reiter, 1987).

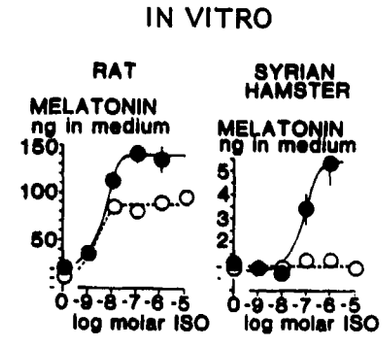

Fig. 2. Mean ± SE response under the same conditions as in Fig. 1, except that pineals were taken for a 4-h incubation with ISO (instead of injection of the animals) at 2000 h (open circles) or at 0230 h (closed circles) (redrawn from Vaughan et al., 1987).

CONCLUDING REMARKS

A day/night change in response of the pineal to β-adrenergic stimulation is present in the Sprague-Dawley rat and is manifested more dramatically in humans and Syrian hamsters which exhibit daytime unresponsiveness of the pineal. This appears not to represent a methodologic artifact either of the protective neuronal uptake phenomenon or of a potential requirement for supplemental alpha activity present in the transmitter, NE. Recent studies (A. Pangerl, B. Pangerl, and R.J. Reiter, personal communication) of iodopindolol binding in Syrian hamster pineals indicated a fall in membrane β-adrenergic receptor function only during the second half of darkness, precisely the time of the endogenous melatonin surge and the sensitive period for stimulation of pineal melatonin by exogenous catecholamine in this species. Light exposure from the beginning of the night not only blocks the fall in membrane receptor binding but also inhibits the development of pineal sensitivity to catecholamine. Development of nocturnal sensitivity may thus represent activation of ligand-bound receptors by ligand dissociation or by internalization and solubilization of the complex. Nocturnal activation of other needed post-receptor elements and lysis of inhibitory elements or molecular structural conformations by lysozymes remain as other possibilities. Whatever its mechanism, daytime unresponsiveness of the pineal contributes to the stability of the melatonin rhythm. Such stability may have implications for the actions of melatonin. These actions may depend on the relative timing of the surge, during which melatonin would be either active or not, depending on other circumstances. For example, environmental conditions which provoke or prevent melatonin-dependent gonadal, thyroidal, and metabolic responses may do so partly by inducing changes in

the cyclic timing of periods of sensitivity to this hormone in target systems (see R.J. Reiter, this volume).

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